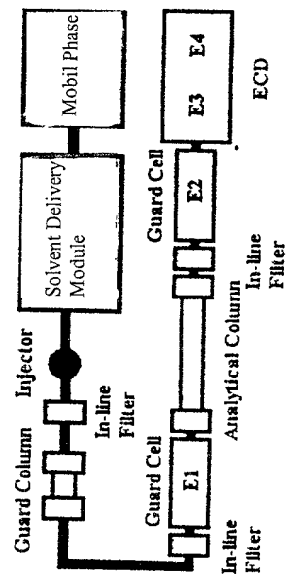


Figure 1

Schematic Diagram of HPLC-EC System



Mobile phase: Sodium acetate 6.8 g
 Acetic acid 15 mL
 Methanol 695 mL
 2-Propanol 15 mL
 Hexane 275 mL

Three operating modes:

1. Mode A measures total reduced CoQ10.
2. Mode B measures total oxidized CoQ10.
3. Mode C measures both reduced and oxidized CoQ10 simultaneously.

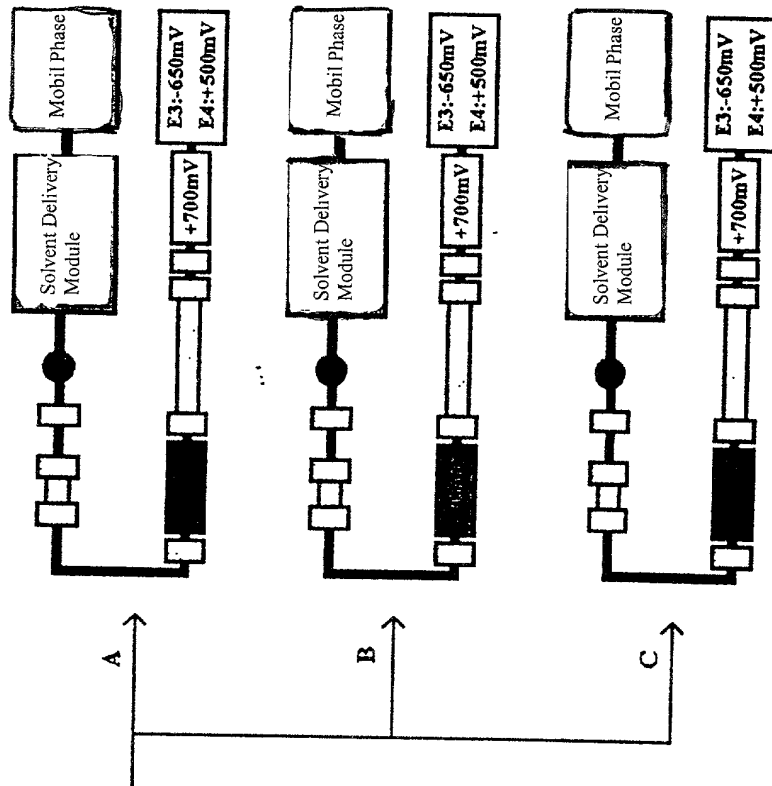


Figure 2.

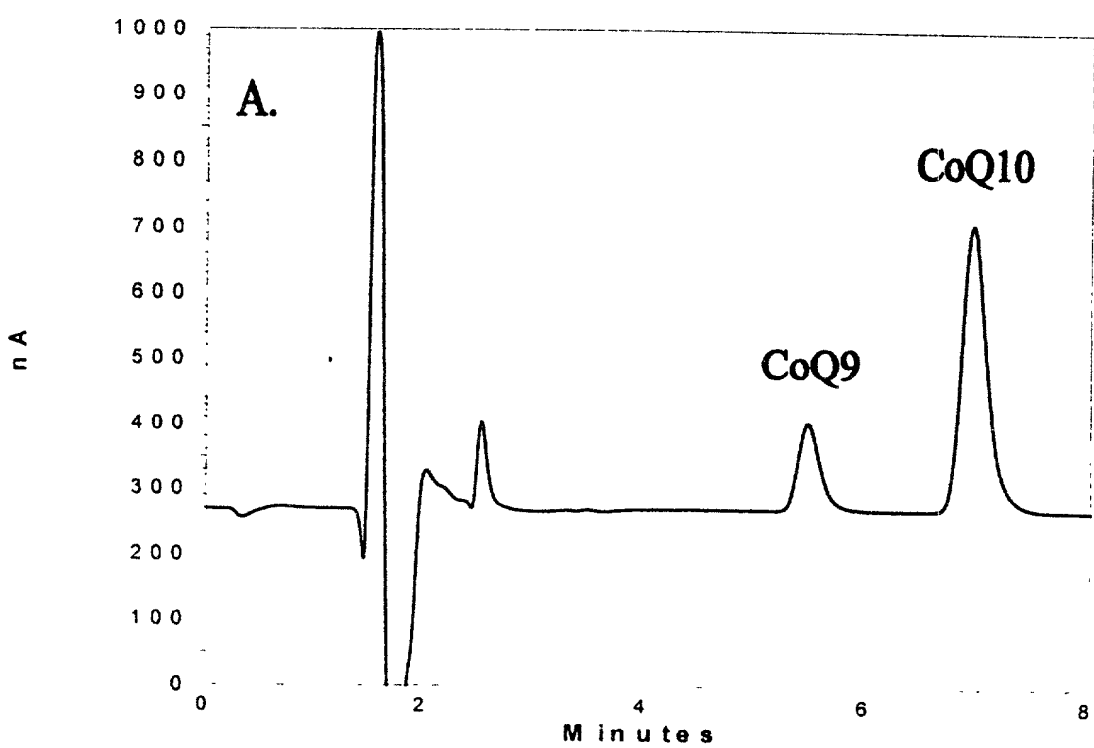
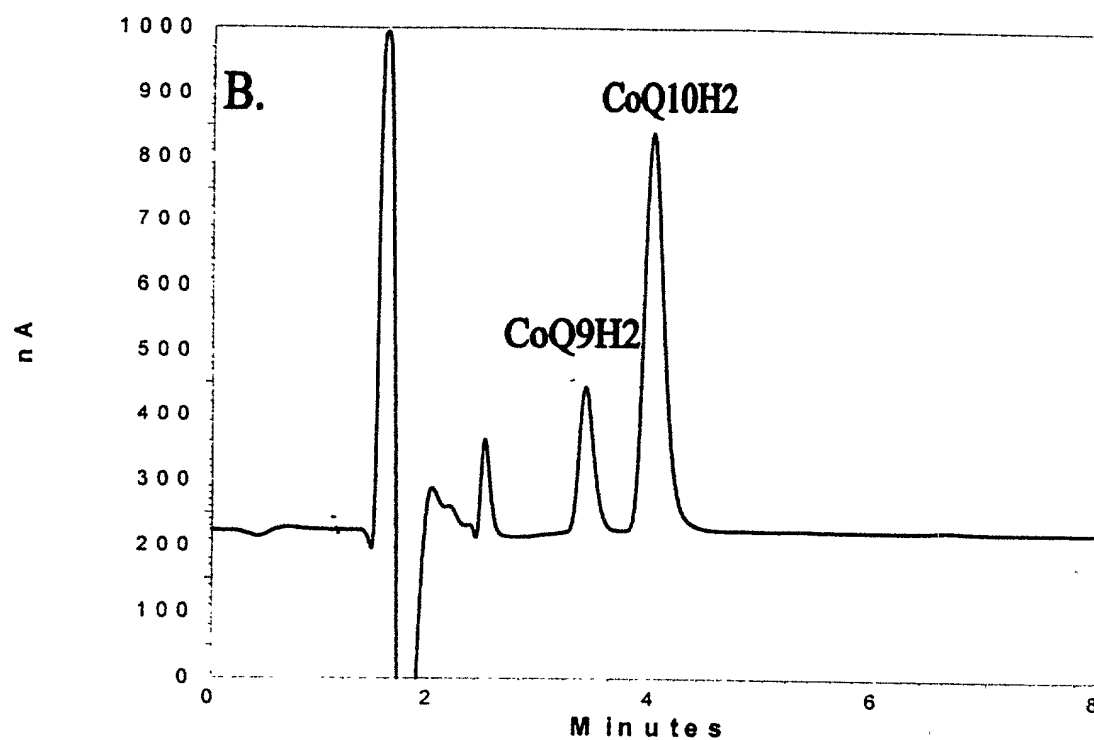


Figure 3.

Calibration Curves for Reduced and Oxidized CoQ10

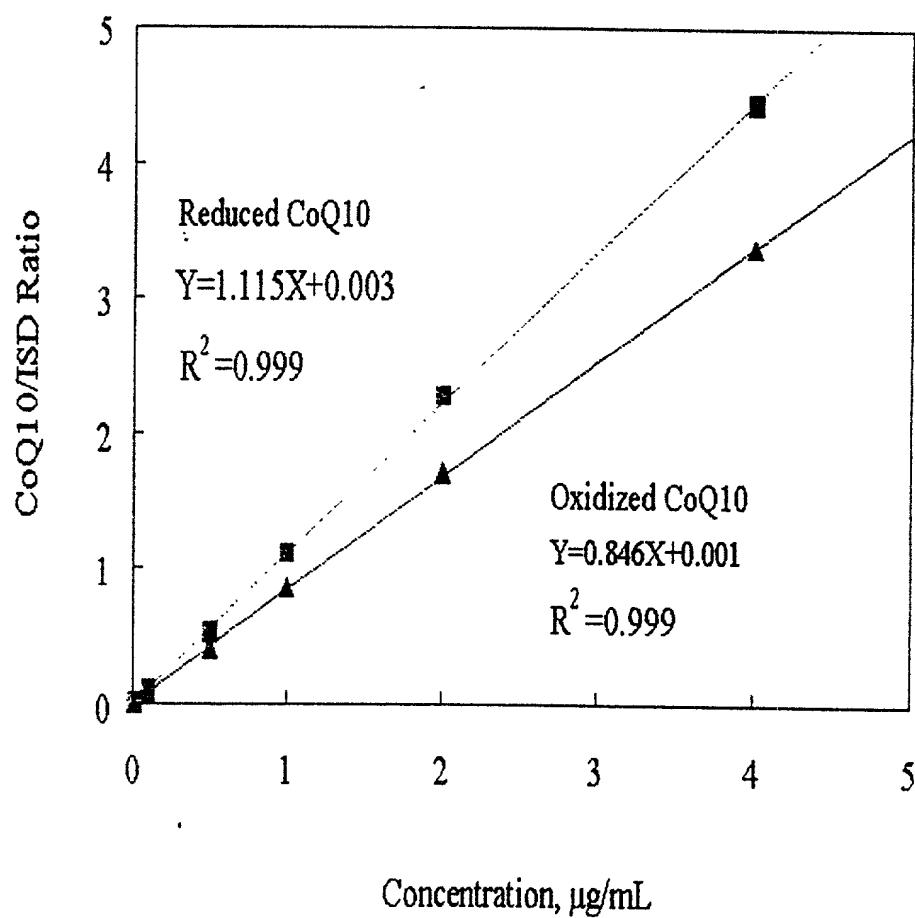


Figure 4.

Effect of Heparin and EDTA on CoQ₁₀H₂ Stability

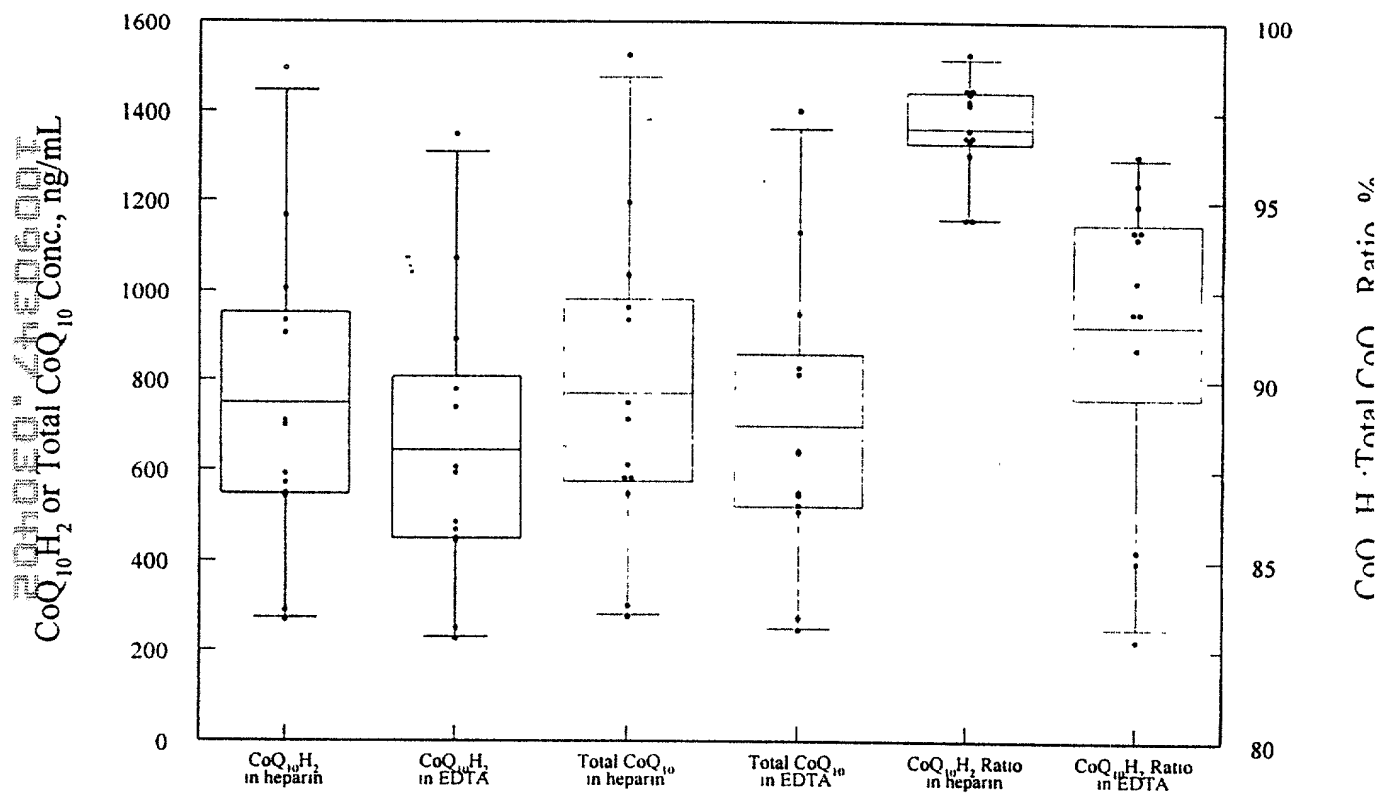


Figure 5.

Comparison Between EDTA and Heparin (Blood Specimen Stored at +4 °C)

△ EDTA ● Heparin □ Heparin Gel

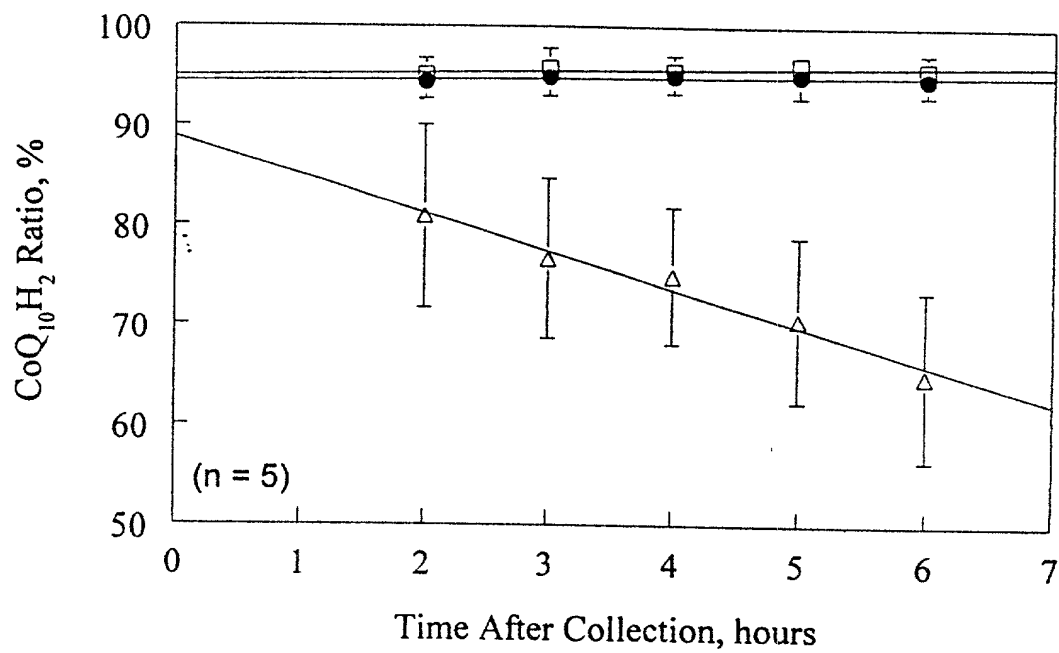


Figure 6.

Stability of $\text{CoQ}_{10}\text{H}_2$ in Heparin Vacutainer®
(Blood Specimens Stored at 4 °C)

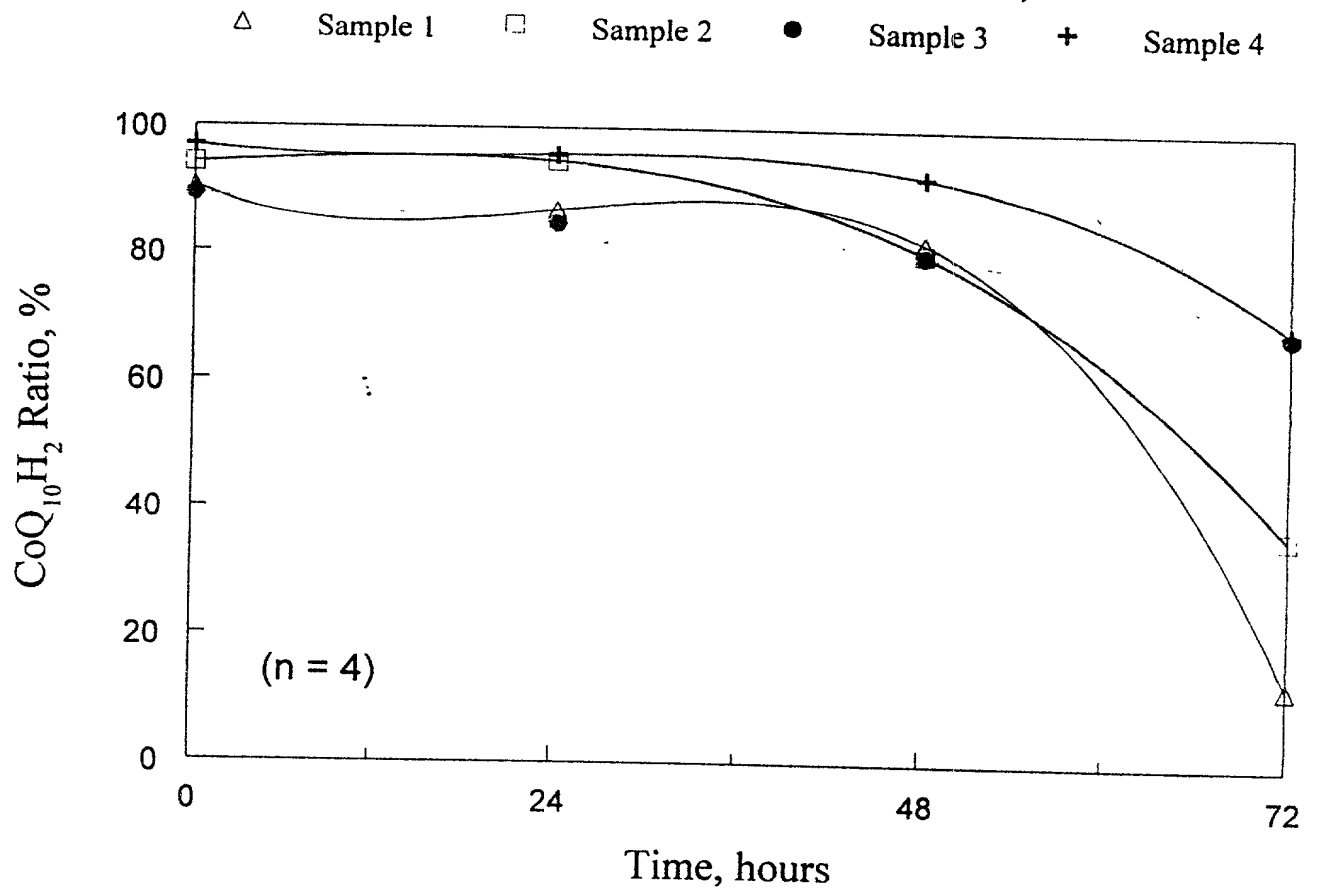


Figure 7.

CoQ₁₀H₂ in Heparin (1-Propanol Extract at 0 °C)

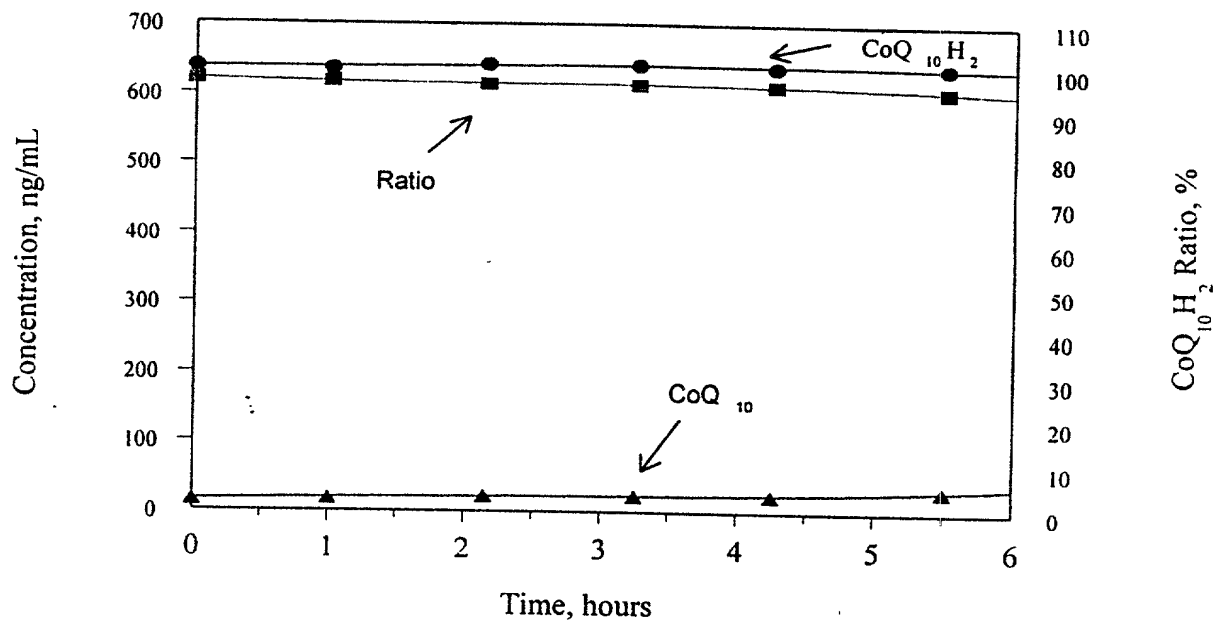


Figure 8.

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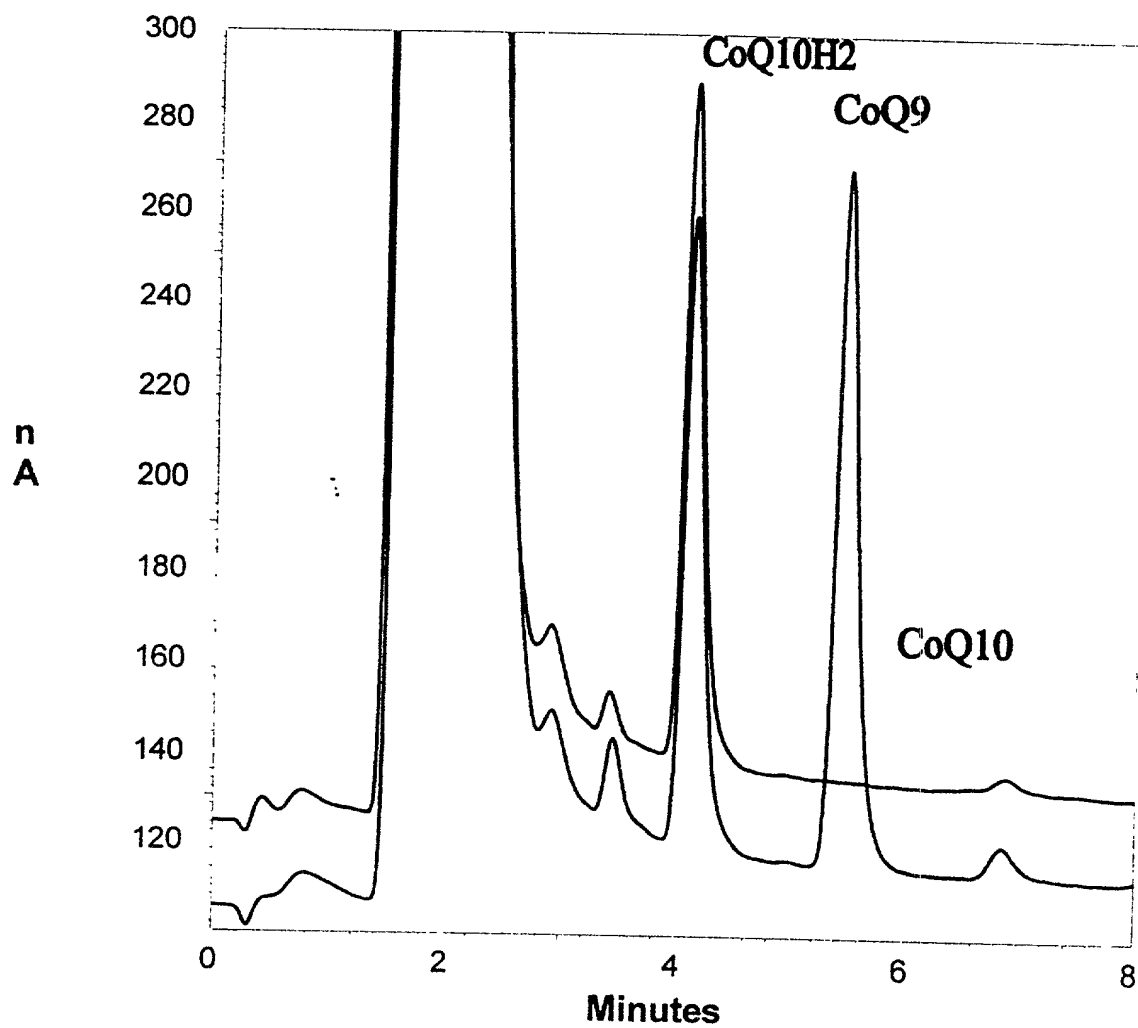


Figure 9.

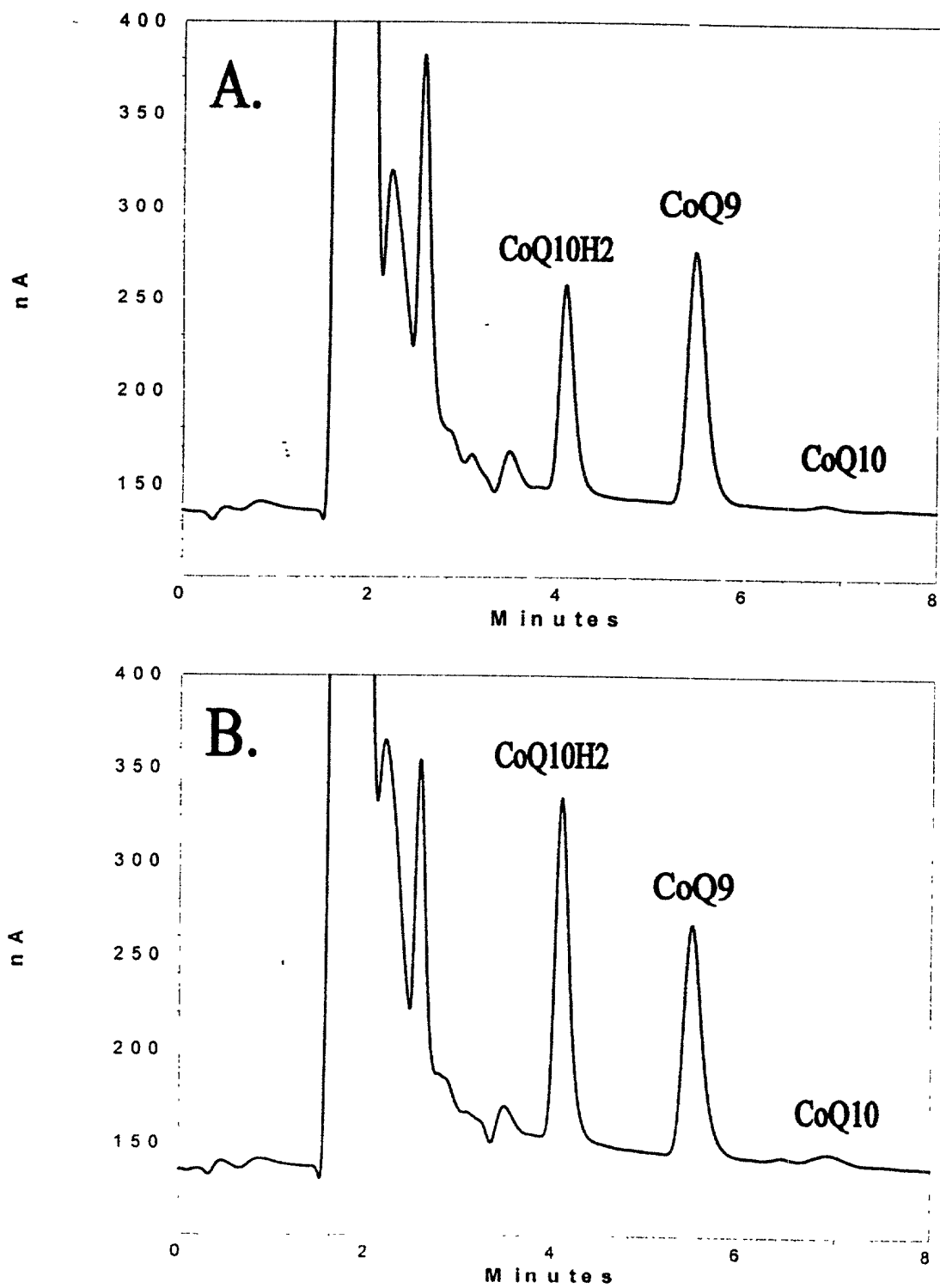


Figure 10.

Table 1. Comparison between current and previous studies on CoQ₁₀H₂ analysis.

Ref. #	Blood Sample	Sample Size, μ L	Extraction Solvent	Precolumn Reduction	Internal Standard	HPLC Run Time, min	CoQ ₁₀ H ₂ Ratio, %	Configuration
1	Heparin	1000	Ethanol/hexane	NaBH ₄	CoQ ₉	10	51.1 \pm 4.2	Amperometric detector
2	Heparin	200	Hexane	Na ₂ S ₂ O ₄	CoQ ₉	14	N/A	Amperometric detector
3	Heparin	300	1-Propanol	NaBH ₄	Diethoxy CoQ ₁₀	11	N/A	Coupled-column/EC-UV
4	Heparin	200	Hexane	NaBH ₄	CoQ ₉	16	87.0 \pm 1.0	Amperometric detector
5	EDTA	1000	Hexane/SPE	EC	CoQ ₉	10	N/A	SPE, column-switching
6	Serum	100	Hexane	NaBH ₄	CoQ ₉	15	65.8 \pm 4.2	Amperometric detector
7	Heparin	10	Hexane	Na ₂ S ₂ O ₄	CoQ ₉	20	93.6 \pm 3.0	Coulometric detector
8	EDTA	300	1-Propanol	NaBH ₄	None	13	94.3 \pm 0.7	Post-column valve switching
9	Heparin	50	Methanol/hexane	NaBH ₄	CoQ ₉	22	91.5 \pm 5.7	Amperometric detector
10	Heparin EDTA	500	Methanol/hexane	EC	CoQ ₇ , CoQ ₉	15-20	88.6 \pm 1.0	Amperometric detector
11	EDTA	100	2-Propanol	NaBH ₄	None	13	~93	Amperometric detector
Current	Heparin	100	1-Propanol	EC	CoQ ₉	8	96.6 \pm 2.2	Coulometric detector

Table 2. Electrochemical reduction of CoQ₁₀

Concentration ng/mL	CoQ10H2 ng/mL	Unchanged CoQ10 ng/mL	Conversion Rate %
10	10	0	100
100	100	0	100
1000	989	11	98.9
1500	1485	15	99.0
2000	1982	18	99.1
3000	2977	23	99.2
4000	3974	26	99.3
(n=2)			Mean = 99.4

Table 3. Extraction recoveries of CoQ₁₀ and CoQ₉ using different solvents

Compound	Extraction Solvent	Volume (μL)	Recovery mean, %	n
		Plasma:Solvent		
CoQ ₁₀	1-Propanol	100:900	100	6
CoQ ₉	1-Propanol	100:900	100	6
CoQ ₁₀	2-Propanol/1-Propanol	100:850:50	89	2
CoQ ₉	2-Propanol/1-Propanol	100:850:50	100	2
CoQ ₁₀	Methanol/1-Propanol	100:850:50	19	2
CoQ ₉	Methanol/1-Propanol	100:850:50	35	2
CoQ ₁₀	Ethanol/1-Propanol	100:850:50	88	2
CoQ ₉	Ethanol/1-Propanol	100:850:50	96	2
CoQ ₁₀	n-Butanol/1-Propanol	100:850:50	85	2
CoQ ₉	n-Butanol/1-Propanol	100:850:50	91	2
CoQ ₁₀	Acetone/1-Propanol	100:850:50	71	2
CoQ ₉	Acetone/1-Propanol	100:850:50	87	2
CoQ ₁₀	Acetonitril/1-Propanol	100:850:50	19	2
CoQ ₉	Acetonitril/1-Propanol	100:850:50	50	2
CoQ ₁₀	Ethanol/Hexane (2:5 v/v)	100:900	98	6
CoQ ₉	Ethanol/Hexane (2:5 v/v)	100:900	100	6

Table 4. Precision Data for CoQ₁₀ analysis.

Intended Conc. ng/mL	Measured Conc. ng/mL	R.S.D. %	Recovery %
Within-Day Precision (n=6)			
75	74±3	3.6	99.3
450	431±11	2.7	95.8
1200	1171±14	1.2	97.6
Day-Day Precision (n=54, 9 days)			
75	76±4	4.9	100.7
450	437±19	4.3	97.1
1200	1174±28	2.4	97.8